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Hypoxic-Ischaemic Encephalopathy and the Blood-Brain Barrier in Neonates

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Abstract
This review aims to highlight a possible relationship between hypoxic-ischaemic encephalopathy (HIE) and the disruption of the blood-brain barrier (BBB). Inflammatory reactions perpetuate a large proportion of cerebral injury. The extent of injury noted in HIE is not only determined by the biochemical cascades that trigger the apoptosis-necrosis continuum of cell death in the brain parenchyma, but also by the breaching of the BBB by pro-inflammatory factors. We examine the changes that contribute to the breakdown of the BBB that occur during HIE at a macroscopic, cellular, and molecular level. The BBB is a permeability barrier which separates a large majority of brain areas from the systemic circulation. The concept of a physiological BBB is based at the anatomical level on the neurovascular unit (NVU). The NVU consists of various cellular components that jointly regulate the exchanges that occur at the interface between the systemic circulation and the brain parenchyma. There is increased understanding of the contribution of the components of the NVU, e.g., astrocytes and pericytes, to the maintenance of this physiological barrier. We also explore the development of therapeutic options in HIE, such as harnessing the transport systems in the BBB, to enable the delivery of large molecules with molecular Trojan horse technology, and the reinforcement of the physical barrier with cell-based therapy which utilizes endothelial progenitor cells and stem cells.

Introduction

The unifying disturbance to neural tissue in hypoxic-ischaemic encephalopathy (HIE) is a deficit in oxygen supply [1]. This can occur because of hypoxia, a diminished amount of oxygen in the blood supply, and ischaemia, a diminished amount of blood perfusing the brain. It is important to recognize that the resulting damage to the brain tissue continues to develop hours to days after the initial HI episode in term newborns [2–4]. Northington et al. [5] propose the concept of "continuum in cell death," whereby the degeneration of neurons lies along a continuum between apoptosis and necrosis. This concept emphasizes the variety of mechanisms of injury and the complex cellular interactions that occur in response to it,

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which ultimately contribute to cell death. After the relative success of translating mild therapeutic hypothermia from the bench to the bedside for neuroprotection, adjunctive treatments which aim to further increase neuroprotection are undergoing exploration.

In this paper, we review the relationship between HIE and the blood-brain barrier (BBB). More than just being a physical barrier formed by tight junctions between the endothelial cells, the BBB is a dynamic physiological barrier which regulates the passage of hydrophilic molecules into the central nervous system (CNS) via transporters and enzymes [6–9]. There is active communication between endothelial cells, pericytes, astrocytes, microglia, the structural basement membrane, and the extracellular matrix (ECM), which is vital for the modulation and the maintenance of the selective permeability of the BBB. Perivascular macrophages, which are adjacent to endothelial cells immediately beyond the basement membrane, also have a regulatory role. This complex system is referred to as the neurovascular unit (NVU) [10, 11], and it underlies the BBB function by acting as an exchange interface between the blood and the CNS. It enables the CNS environment to remain stable despite fluctuations in the composition of plasma and brain interstitial fluid, thus preventing interference with signal transmission. The progress in the characterization of the BBB structure through proteomics [12] and the improvement in drug delivery by taking advantage of the endogenous transporting systems of the BBB [13, 14] hint at the potential of the BBB to take a more prominent role in our understanding and management of HIE. However, this discussion also serves to highlight the challenges and controversies in our understanding of the BBB and its relationship to HIE. The observations discussed in this review originate from a variety of experimental models (summarized in Table 1).

Defining the Blood-Brain Barrier by Its Morphology and Function

A cross-section of the NVU can be visualized as a layer of endothelial cells and interconnected junctional-protein complexes surrounded on the outside by a basement membrane which is shared with enveloping pericytes. Perivascular macrophages abut endothelial cells. Beyond the basement membrane, astrocytic end feet are in direct communication with the vascular lining and thus act as a cellular link with neurons, all of which are anchored in place by ECM proteins [6]. The significant restriction of passage of substances across the BBB is reflected in the high trans-endothelial electrical resistance (TEER) across the endothelial cells of the BBB, which is 50 times higher than that across the peripheral endothelium [15]. One of the main physical characteristics of the brain endothelial lining, underpinning its barrier function, lies in the complex tight junctions which limit the intercellular movement of molecules [7]. Amongst the proteins making up this structure are the transmembrane proteins occludin and claudin [16]. There is evidence that claudins contribute to the high TEER across the BBB [7], and claudin-5 shows promise as a therapeutic target as BBB permeability to small molecules of a molecular weight of <800 Da increases in its absence [17]. The role of other proteins, including zonula occludens proteins (ZO-1, ZO-2, and ZO-3), which anchor the tight junctions to the cellular cytoskeleton, and junctional-adhesion molecules (JAM-A, JAM-B, and JAM-C), which help regulate the tightness of the junctions, is not as well understood and requires further work [18].

The Role of Pericytes

Platelet-derived growth factor BB (PDGF-BB), secreted by the endothelial cells, forms a concentration gradient at the basement membrane and recruits pericytes to take up their position [19]. Pericytes reinforce and support the NVU; in their absence, microaneurysms form and there is rupture of the microvascular structures [20, 21]. Pericytes may be important in determining BBB permeability, by inhibiting the expression of molecules promoting vascular permeability and immune cell infiltration. Pericytes regulate functional aspects of the BBB, including the formation of tight junctions and vesicle trafficking in CNS endothelial cells [22].

The Role of Astrocytes

The role of astrocytes in the BBB is less certain and is more controversial [23–25]. Studies using immunohistochemistry and co-cultures of astrocytes with endothelial cells and pericytes show that astrocytes are necessary to guide interactions between these cells, and their presence contributes to the upregulation of BBB properties in the endothelial lining [26]. It has been postulated that because astrocytes appear in development after the vascularization of the brain, this emphasizes their "maintenance" and supportive role.

The Role of CNS Mononuclear Phagocytes

Microglia are the most abundant myeloid cell population in the CNS. Determining the origins of microglia has

Table 1. Summary of studies on the BBB and the models on which they were based

First author [Ref.], year	Experimental model
Colbourne [3], 1995	15-week-old gerbils; temporary occlusion of carotid arteries
Geddes [4], 2016	P7 rats; carotid artery ligation and hypoxia
Butt [15], 1990	infant and fetal rats (age range: 17 gestational days to P33); hyperosmotic shock and metabolic poisons
Nitta [17], 2003	genetically modified claudin-5-deficient newborn mice
Lindahl [20], 1997	PDGFB-deficient mouse embryos
Tallquist [21], 2003	PDGFR-β allelic variants – mouse embryos
Janzer [24], 1987	neonatal rat astrocytes and meningeal cells
Ramsauer [26], 2002	astrocytes from rats aged 8–12 weeks and 1–2 days; endothelial cells and pericytes
He [29], 2016	mice aged 8–12 weeks
Wang [30], 2012	genetically modified mice (Frizzled signalling) ranging from newborns to adults
Liebner [31], 2008	genetically modified mice (Wnt signalling) ranging from embryonic to early postnatal mice + young adult mice endothelial cell culture
Alvarez [32], 2011	human BBB endothelial cells + genetically modified mice (Hedgehog signalling) ranging from embryos to adults
Haqqani [36], 2013	immortalized human brain microvascular endothelial cells
Grontoft [37], 1954	post-mortem of legally aborted human and rabbit fetuses
Dziegielewska [38], 1979	fetal sheep; early and late gestation permeability studies
Ek [40], 2015	P9 mice; HI model
Muramatsu [41], 1997	P7, P14, and P21 rats; HI model
Chen [42], 2012	fetal sheep ischaemia model
Chen [43], 2006	mammalian cancer cells
Baskaya [44], 1997	adult rats; traumatic brain injury
Kumar [45], 2008	term neonates; HIE
Kotter [49], 2006	adult rats; demyelination model
Ritzel [52], 2015	mice aged 10–12 weeks; middle cerebral artery occlusion
Hu [53], 2012	mice aged 10–12 weeks; focal transient cerebral ischaemia + embryonic rat cell culture
Breckwoldt [54], 2008	adult mice; unilateral middle cerebral artery occlusion
Gliem [55], 2012	mice aged 6–10 weeks; photothrombosis and transient middle cerebral occlusion
Chu [56], 2015	mice aged 8–12 weeks; occlusion of the middle cerebral artery
Crane [57], 2014	mouse aged 8–10 weeks; sterile wound model
Noda [58], 2011	mouse embryonic neuronal and microglial cells
Elliott [59], 2009	mice aged 8–12 weeks; murine air pouch model, mouse aged 4–5 weeks; thymic clearance model
Feng [62], 2008	P7 rats; HIE
Schmid-Brunclik [63], 2008	newborn rat astrocytes
Wang [64], 2001	brain microvessel endothelial cells from bovine grey matter
Kaur [65], 2006	adult rats; hypoxia model
Chen [67], 2008	P7 rats; HI model
Sheldon [68], 2009	P7 mice; hypoxia and HI model
Nedelcu [69], 1999	P7 rats; HI model
Manley [74], 2000	adult mice; AQP4 deficient and middle cerebral occlusion
Fu [76], 2007	P3 rat astrocytes
Heo [78], 1999	adolescent baboons; middle cerebral artery occlusion
Shankaran [80], 2005	newborns (at least 36 weeks' gestational age); HIE
Azzopardi [81], 2009	newborns (at least 36 weeks' gestational age); perinatal asphyxia
Baumann [82], 2009	adult rats; bilateral common carotid artery occlusion
Jurkovich [83], 1988	cat intestine; ischaemia model
Boado [86], 2010	adult rhesus monkey; BBB and fusion protein studies
Boado [87], 2010	adult rhesus monkeys; BBB and fusion protein studies
Zhou [89], 2011	adult mice; 6-hydroxydopamine model of Parkinson disease
Sumbria [90], 2012	adult mice; reversible middle cerebral artery occlusion
Sumbria [91], 2013	adult mice; reversible middle cerebral artery occlusion
Liao [92], 2013	neonates with HIE and children with cerebral palsy
Asahara [94], 1997	human endothelial progenitor cells; rabbit and mouse ischaemia models
Peichev [95], 2000	human fetal liver cells and cord blood cells
Zhang [97], 2002	adult mice; middle cerebral artery occlusion
Fan [99], 2010	adult mice; transient middle cerebral artery occlusion
Ohta [100], 2006	rats aged 8–10 weeks; middle cerebral artery occlusion

P, postnatal day.

been challenging due to their unique characteristics as both glial cells involved in regulating synaptic structures and immune cells surveying and responding to injury. Microglia are derived from haematopoietic stem cells in the yolk sac, differing thus from peripheral macrophages, which are derived from the bone marrow [27]. Embryonic microglia actively proliferate, maintaining a steady-state supply into adulthood, without a contribution from peripheral monocytes. Microglia are present in the CNS prior to the endothelial presence, and it has been suggested that the interaction between microglia and endothelium plays a key role in BBB formation and function. Changes in equilibrium associated with injury, however, result in the recruitment of peripheral monocytes which are able to differentiate into cells with properties similar to those of microglia (also called monocyte-derived macrophages, MDMs). The contribution of the embryonic or peripheral MDMs to the acute and chronic phases of HI injury has yet to be fully established.

Other mononuclear phagocytes that exist in the CNS, e.g., the perivascular macrophages which contribute to the NVU, have less well-defined roles. They originate from the bone marrow and reside in closer communion with the vasculature as opposed to the microglia, which infiltrate the CNS tissue [28]. A recent study [29] has revealed that communication with endothelial cells appears to promote polarization of the perivascular macrophages to exhibit an M2 phenotype (a concept discussed later). Indeed, it appears that only peripheral monocytes expressing genes favouring differentiation to the M2 phenotype are able to reconstitute previously disrupted BBB permeability. Furthermore, similarly to pericytes, perivascular macrophages can dissociate from the vasculature and contribute to the increased permeability of a disrupted BBB [29].

The BBB as a Dynamic Barrier

The brain endothelial cells express BBB-specific genes that enable the development of the endothelial barrier in the absence of external influences. However, pericytes, astrocytes, perivascular macrophages, and microglia may still have a role to play in regulating and maintaining the functional characteristics of the BBB after the formation of the endothelial barrier. Recent studies show that the BBB is not an unassailable structure and that removal of the interdependent components of the NVU can result in the loss of its barrier properties [30–32].

The BBB is a functionally dynamic construct. For example, endothelial dysfunction is associated with the release of microparticles such as endothelial microvesicles and exosomes. These particles contain mixtures of proteins and other components, which are shared with the parent cell [33]. They have a diverse profile of adhesion molecules, antigen-presenting molecules, and other proteins involved in cell-to-cell signalling, and have been a recent focus of interest for their proposed role in the cellular and BBB trafficking of molecules [34]. Particularly of interest is their specificity and sensitivity as biomarkers of the involvement of the BBB in neurological pathologies that can be detected peripherally, as they are extruded from the brain endothelial cells into the bloodstream [35, 36].

Maturation of the BBB

The developmental changes in BBB morphology and function are not fully understood and much of our current understanding of the effect of hypoxia on the cerebral vasculature is derived from adult models.

In 1954, Grontoft et al. [37] demonstrated that the BBB obtained from legally aborted human fetuses was impermeable to Trypan blue dye as early as at the point of placenta separation. Their interpretation of this observation was that a functional BBB was already present at the fetal stage of human development. In addition, brain staining was noted to occur when some time had elapsed after placental separation. This was postulated, and later confirmed, to be the effect of hypoxia on the permeability of the BBB [23]. There is evidence that the fetal and newborn BBB may indeed be functional, at least in lower species; immunostaining reveals evidence of a functionally intact BBB in rats as early as embryonic day 16 (E16) [23].

In E60 fetal sheep, Dziegielewska et al. [38] noted that the intravascular injection of Alcian blue had reached the brain tissue, not through the tight junctions, which were observed to be well formed by E60, but via intracellular vesicles, with declining activity by E125. Thus, the idea was conceived of the earlier maturation of tight junctions, followed by the maturation of transcellular transport in the developing BBB. These 2 mechanisms have been proposed to become fully functional at different points along the timeline of development.

The Effects of HI Injury on the BBB

Important effects of HI injury on the BBB include angiogenesis and changes in permeability. Several key molecules have a role in angiogenesis, including hypoxia-

Table 2. Summary of studies on the timeline of BBB permeability changes after HI injury

First author [Ref.], year	Animal model	Age	Measured indicator of BBB permeability	Earliest peak of BBB permeability	Sampling time after HI injury
Muramatsu [41], 1997	rat	postnatal day 7	IgG immunoreactivity	6 h	3, 6, 9, 12, 18, and 24 h
Chen [42], 2012	sheep	125–129 gestational days (85–87% of gestation)	calculation of blood-to-brain transfer constant for radioactive tracer	4 h	4, 24, and 48 h
Ek [40], 2015	mouse	postnatal day 9	CSF-to-plasma sucrose concentration ratio and albumin immunoreactivity	2 h	2, 6, 24, and 72 h (and 168 h for sucrose concentration ratio)

inducible factor-1 ($HIF_{1\alpha}$), vascular endothelial growth factor (VEGF) and erythropoietin. Angiogenesis can be divided into 2 processes: angiogenic remodelling and angiogenic sprouting [39]. The latter involves the replacement of old vessels, which are broken down, with new immature vessels, leaving the BBB vulnerable to oedematous disruption and mechanical stress. This is in comparison to the former, whereby existing vascular networks undergo changes leading to the development of a mature, stable vasculature contributing to the BBB.

BBB permeability has been measured by various methods and the models used to study permeability in response to HI insult have been diverse. There is consensus that there is an increased early BBB permeability, peaking at between 2 and 4 h after insult in most neonatal injury models (Table 2) [40–42].

In the neonatal injury models, there is less compelling evidence of a delayed second phase of increased BBB permeability, which has been noted in the adult rat model [43, 44]. The BBB in human babies with HIE shows increased permeability as assessed by comparing the concentrations of albumin in cerebrospinal fluid (CSF) versus plasma [45]. Equally as important, this study also lends support to the proposed relationship between HIE and the BBB, suggesting free radical injury as an amplification factor in the pathophysiological processes of both HIE and BBB dysfunction.

Whether or not the BBB experiences a second phase of increased permeability after HI injury, there is early activation of mechanisms that lead to the eventual restoration of BBB function after the insult, as demonstrated by the observation of an upregulation in the transcription and expression of tight-junction proteins in the neonatal mouse and the fetal sheep model [40, 42].

The Effects of HI Injury on the Cellular Components of the NVU

Engelhardt et al. [46] demonstrated that the endothelial cells are more susceptible to hypoxia-induced injury, as seen by a disruption in their cellular cytoskeleton structure, than pericytes and astrocytes which retain the cytoskeleton arrangement even with prolonged hypoxia. This was discussed in the context of the oxygen concentration these cells are exposed to in the normal brain environment, whereby astrocytes and pericytes occupy areas exposed to lower oxygen concentrations than the endothelial cells. The case becomes more complex when it is noted that the proliferation of pericytes and astrocytes is downregulated when exposed to hypoxia whereas endothelial cells continue to proliferate despite this being counterproductive to the conservation of energy and, ultimately, their survival. For endothelial cells, this is further associated with a rapid induction of the HIF_1 protein and BBB disruption.

Microglia are the first immune cells of myeloid origin to respond to signals of inflammation such as damage-associated molecular patterns (DAMPs) released from damaged tissue [47]. Their role remains controversial. Microglia may also have a neuronal protective role against toxicity associated with stimulation of the NMDA glutamate receptors, as demonstrated by studies introducing microglia to previously microglia-free, organotypic slice cultures [48].

Microglia phagocytic activity removes cell debris, which is crucial to set the stage for axonal regeneration and structural remodelling of neural networks; myelin remnants may contribute to disruption of remyelination due to the release of growth inhibitors [49]. Furthermore,

their clearance is influenced by the presence of pro-inflammatory cytokines such as TNFα, which have been shown to reduce the phagocytic activity of macrophages [50].

In parallel to the T helper 1 (Th1)/Th2 polarization concept relevant to T cells, macrophages may also be differentiated into an M1/M2 phenotype [51], an oversimplified concept that extends to the microglia as well. M1 macrophages, which are pro-inflammatory, contribute to generating free radicals and matrix metalloproteinase (MMP)9-mediated injury [47]. M2 macrophages promote the resolution of the inflammatory phase and its subsequent transition to healing and repair. In the presence of ischaemia, microglia and MDMs seem to favour differentiation into the M1 phenotype [52], which is associated with worse oxygen-glucose deprivation (OGD)-induced neuronal loss [53]. Peripheral monocytes respond relatively later than microglia, i.e., on days 3–7 after ischaemic stroke [54]; those expressing genes favouring conversion to the M1 phenotype arrive earlier than those expressing genes favouring M2 polarization. Though their pro-inflammatory repertoire seems an obvious threat to mounting injury, their presence has been shown to be vital to the damage control of the ischaemic injury [55]. This may be explained by their ability to influence surrounding microglia/MDMs to convert into M2 phenotype macrophages [56] in addition to their own subsequent conversion to M2 phenotype macrophages. The players controlling the switch from the inflammatory phase to the repair phase, though yet to be defined, are probably a combination of factors. Firstly, M1 macrophages release TGFβ and VEGF once in the area of injury [57]. Secondly, dying cells release CX3CL1, which attracts M2 macrophages, mainly since they have a higher level of expression of corresponding receptors [58–60]. Both of these are associated with wound healing and tissue remodelling.

The Role of VEGF

Not only are the pericytes and astrocytes better able to adapt to the HI insult, they have also been shown to be vital in reducing injury through the secretion of protective factors, such as VEGF. VEGF exists as a family of 6 homologous members: VEGF-A, -B, -C, -D, and -E, as well as placental growth factor [61]. They bind to tyrosine kinase receptors known as VEGF receptors, and are prominently involved in coordinating the development and regulation of blood vessels. The protective role of VEGF lies in increasing the survival of endothelial cells and reducing the volume of infarcted tissue via the inhibition of apoptotic mechanisms [62, 63].

However, VEGF contributes at the same time to increased BBB permeability via the destabilization of junctional proteins, including the tight junctions and adherens junctions [64]. This leads to vasogenic oedema, and may provide the link between increased BBB permeability and the pathophysiological inflammation in HIE [65]. Due to the diversity of the VEGF family, it is possible that the conflicting roles of VEGF following HI brain injury can be explained by the interactions between the different forms of VEGF and their corresponding receptors.

One of the important contributors to VEGF upregulation is $HIF_{1\alpha}$ [66]. Hypoxia initiates the activation of this factor, resulting in the expression of genes responsible for angiogenesis. Similar to VEGF, Baburamani et al. [39] report conflicting effects of $HIF_{1\alpha}$ subsequent to HI insult and suggest that time post-injury is a determining factor. Acutely, the absence of $HIF_{1\alpha}$ has been associated with a reduced degree of injury [67]. Over a longer time course, however, mice unable to express $HIF_{1\alpha}$ were found to have a worse outcome than wild-type controls [68].

A Reversal of Roles: Impact of BBB Dysfunction on the Pathophysiology of HIE

Ek et al. [40] have shown a correlation between areas of disrupted BBB function and areas of infarction in the brain. Nedelcu et al. [69] noted that a moderate HI insult in the brain of postnatal day 7 (P7) rats produced a first phase of cytotoxic oedema, which was followed 4 h later by a second wave of cytotoxic oedema and new development of vasogenic oedema. Cerebral vasogenic oedema develops in the presence of increased BBB permeability, allowing for an unregulated accumulation of water content in brain tissue [70].

The expression of aquaporin 4 (AQP4), the major water channel of the mammalian brain [71], appears to be concentrated at the end-feet of the astrocytic component of the NVU [72]. AQP4 does not act solely as a regulator of water flow, it also serves other physiological functions [73]. AQP4 may control the flow of water bidirectionally, due to the observation that its absence can have 2 opposing outcomes in different types of pathology [74, 75]. The absence of AQP4 reduces the severity of the oedema during the acute phase of HI injury. However, when oxygenation is reintroduced, the absence of AQP4 results in a longer time taken to clear the excess water [76].

The increase in BBB permeability may contribute to HI injury of the brain through the increased exposure of brain tissue to inflammatory mediators. One of the downstream pathways linked to TNFα, which is released in HIE, involves the activation of MMPs [77]. These proteolytic enzymes, especially MMP3 and MMP9, have been implicated in BBB disruption due to proteolysis of ECM proteins and cleavage of tight junctions, resulting in oedema and haemorrhage [78, 79]. Therapeutic hypothermia (TH) is the only effective treatment in clinical use at present that intervenes at the level of the pathological mechanisms of HIE. In the clinical setting, TH has been shown to be effective when commenced within 6 h after birth [80, 81]. From the point of view of the BBB, TH has been shown to reduce the activity of enzymes, especially that of MMPs, as well as maintain ECM molecular and cellular integrity [82, 83].

Exploitation of Molecular Exchanges across the BBB to Increase Drug Delivery to Injured Brain Parenchyma

An early increase in BBB permeability has been noted after HI insult, and we also know that neuroprotective interventions are more likely to be effective if commenced early. Therefore, we may postulate there to be a time window, as yet not clearly defined, in which effective drug delivery through the BBB may optimally exert a protective effect. The second concept is the targeting of changes in the BBB and how we can modulate these in an attempt to repair the injury caused in HIE.

The BBB has, at times, been mistakenly regarded as one and the same as the blood-CSF barrier [84]. While the BBB is spread across the capillaries of the brain, the blood-CSF barrier is focused at the choroid plexus in the ventricles. In the attempts to overcome the restrictions of the BBB, the solution of utilizing the blood-CSF barrier for the delivery of drugs into the intrathecal compartment was developed. However, this mode of delivery is limited by differentials in diffusion rates; diffusion from the CSF into the bloodstream is faster than direct diffusion from the CSF into brain tissue, so, paradoxically, drugs introduced into the CSF compartment are more likely to reach the brain parenchyma via the bloodstream and across the BBB, rather than directly by passage into the brain tissue.

MTH Technology: The Potential Role of Carrier-Mediated and Receptor-Mediated Transporters

The BBB has been considered as the single most important limiting factor in the development of neurotherapeutic drugs [13]. This is partly because reliance on high-throughput screening (HTS) is more likely to select molecules of high molecular weights. Hydrophobic molecules with a molecular weight of <400 Da cross the BBB by free diffusion, while hydrophilic and large molecules gain access either via the carrier-mediated or receptor-mediated transport (RMT) system [84].

These systems transport molecules which retain the generic structures of the original substrates which the carriers and receptor transporters recognize as their target. This concept has led to the utilization of the intrinsic mechanism of the BBB to enable drug delivery in the form of molecular Trojan horse (MTH) technology. This relies on combining biologic therapeutics with monoclonal antibodies (MAbs, which are specific for receptors across the BBB), which take up substances from the periphery via RMT systems, such as insulin and transferrin [14, 85]. Two of these genetically engineered MAbs are fusion proteins with IgG portions targeted against the human insulin receptor (HIR) and transferrin receptor (Tfr), i.e., HIRMAb and TfrMAb [85].

At present, all the evidence for the effectiveness of this technology is extrapolated from adult models. The final consensus on the functional maturity of the BBB in the fetus and the newborn has not been reached. On an optimistic note, inward transport, including the RMT for transferrin, across the BBB has been shown to function in the fetal and newborn brain [23].

The utility of MTH has enabled large molecules such as TNFα inhibitors [86] and erythropoietin [87] to be delivered across the BBB. We use these examples as agents being investigated for the treatment of HIE [88]. They have been shown to fulfil several criteria for effective MTH technology, including having a higher affinity of the fusion protein than the MAb ~~only~~ for its target [89] as well as having a higher uptake across the BBB via a higher affinity for the specific RMT system [86].

Most studies of MTH technology have focused on the effect of these fusion proteins in adult models of acute stroke when assessing the impact on pathology, and they have demonstrated a reduction in infarct volume [90, 91]. Given that a number of the pathophysiology mechanisms are the same as those seen in stroke provides hope that this technology may be applicable not just to HIE but also

neonatal encephalopathy from other causes. These studies await translation from the laboratory models to the clinic.

Stem Cell Therapy

Another exciting innovation that builds upon the concepts of the synergy of action between the different cells of the NVU is cell-based therapy. While deriving new cells from stem cells and progenitor cells is surrounded by much controversy, it remains, in theory, an attractive proposal for the repair and prevention of further injury to the brain. Cell-based therapies can be derived from various sources including umbilical cord blood, bone marrow, embryonic nervous tissue, and even adult brains [92]. Of interest in the BBB and indeed the nervous system, stem cells from umbilical cord blood are considered to be advantageous because they are a rich source of endothelial progenitor cells (EPCs) [93], cells which have just begun to change our understanding of vascular development, injury, and repair.

EPCs are a population of stem cells which are activated and recruited from sources including the bone marrow, umbilical cord blood, and peripheral blood, in reaction to hypoxia [94, 95]. Their discovery led to the new concept of vasculogenesis [96]; this is the development of new blood vessels by the recruitment and maturation of EPCs, in contrast to angiogenesis whereby new blood vessels are formed from proliferation of the pre-existing endothelium. Thus, it was hypothesized that harnessing the potential of EPCs in promoting neovascularization in the presence of ischaemic injury [97] would be valuable as a novel therapeutic intervention [98]. Indeed, several studies which have begun to assess the impact of the activation of EPCs in the presence of ischaemic injury have demonstrated that EPC treatment reduces infarct volume, improves deficits in cognitive learning and motor skills, and is associated with the recovery of regional cortical blood flow [99, 100].

However, as a note of caution: EPC treatment has been linked with the production of nitric oxide and VEGF [101]. While the properties of EPCs as growth factors and vasodilators may contribute to the restoration of blood flow to the ischaemic regions, a consensus on the effect of restoring cerebral blood flow and its impact on HIE has yet to be reached. Furthermore, these molecules have also been associated with BBB breakdown as well as the excitotoxic damage noted in HIE. The huge potential of EPC therapy has to be balanced with a need for the in-depth study of its potential risks and the harmful contributions it may make to the pathways of HI injury.

Conclusion

In this review, we started with our understanding of the structure and function of the BBB. We went on to discuss the effects of HI injury of the immature brain on the BBB, and finally we touched on exciting therapeutic options linked to the targeting of the BBB, including MTH technology and stem cell therapy. These developments offer new hope for the improved management of HIE in the future.

Disclosure Statement

None of the authors has any conflict of interest to declare.

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